JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
MURUGAN S *, MANI KR**, UMA DEVI P***Prevalence of Methicillin Resistant Staphylococcus Aureus among Diabetes Patients with Foot Ulcers and their Antimicrobial Susceptibility Pattern. Journal of Clinical and Diagnostic Research [serial online] 2008 August [cited: 2008 August 14]; 2: 979-984.
Available from
http://www.jcdr.net/back_issues.asp?issn=0973-709x&year=2008&month=August &volume=2&issue=4&page=979-984 &id=258

ORIGINAL ARTICLE

Prevalence of Methicillin Resistant Staphylococcus aureus among Diabetes Patients with Foot Ulcers and their Antimicrobial Susceptibility Pattern

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ABSTRACT

Background and Objectives: MRSA infection is common in diabetes patients with foot ulcers, and is associated with previous antibiotic treatment and prolonged healing. As only a few studies with a small number of samples were done in this regard in India, the present study was undertaken to report the prevalence and antibiotic susceptibility pattern of MRSA screened from diabetes patients with foot ulcers, attending tertiary care hospitals.

Settings and Design: It was a retrospective study carried out at Department of Microbiology, Dr.N.G.P Arts and Science College, Coimbatore, Tamil Nadu, India during the period from November 2002 to October 2006.

Methods: The present study comprised of 7354 pus specimens of diabetic foot ulcer patients collected from various tertiary care hospitals in and around Coimbatore. All the samples were subjected to gram staining and bacterial culture, and the *S. aureus* isolates were screened for MRSA prevalence using conventional microbiological methods. Subsequently, the antibiotic sensitivity test was performed for the confirmed MRSA isolates.

Results: Out of 2314 (37.82 %) strains of *S. aureus* isolated form diabetic foot ulcers, 992 (42.86 %) were found to be methicillin resistant. More precisely, all MRSA strains (100 %) were resistant to penicillin, 90.92 % to ampicillin, 82.76 % to clotrimoxazole, 64.11 % to gentamicin, 60.08 % to erythromycin, 51.91 % to omnatax and 50.10 % to cephalexin. Multi drug resistance for about 7 to 10 antibiotics was observed among 55.0 % of the isolates. However, all the strains were sensitive to vancomycin (100 %).

Conclusions: The determination of prevalence and antibiotic sensitivity pattern of MRSA screened from diabetic foot ulcer patients will help the clinician for first line treatment in tertiary care hospitals.

Key Words: Diabetes patients, foot ulcers, MRSA, prevalence, multi drug resistant, S. aureus.

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Introduction

Diabetes currently affects more than 194 million people worldwide, and the figure is expected to reach 333 million by 2025, with the maximum burden falling upon developing countries. India, considered as the "diabetic capital of the world", alone, currently accounts for over 35 million people harboring diabetes[1]. This number is estimated to touch 73.5 million by 2025, as a consequence of longer life expectancy,

sedentary life style and changing dietary patterns[2]. Diabetes is a chronic disorder and affects a large segment of the population. Diabetes and foot problems are almost synchronous[3]. As many as 25 % of diabetic individuals are expected to develop severe foot problems at some point in their lifetime, which often leads to amputation [4]. Diabetic foot infections are more severe and more difficult to treat than infections in non-diabetics. Staphylococci and β-haemolytic Streptococci are the most commonly isolated pathogens in moderate and severe diabetic foot infections. Aerobic gram-positive cocci are the predominant microorganisms that colonize and acutely infect breaks in the skin.[5] The genus Staphylococcus includes pathogenic organisms in which *S. aureus* is the most important one that has become resistant to most of the therapeutic agents that have been developed in the recent years, and hence the antimicrobial chemotherapy for this species has always been empirical[6]. *S. aureus* is the most important pathogen in diabetic foot infections, even when it is not the only isolate; it is usually a component of a mixed infection[5]. The most notable example of this phenomenon was the emergence of methicillin resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the launch of methicillin[7]. Overuse of antibiotics and the selection of broad, rather than narrow spectrum agents, have contributed to the high prevalence of methicillin- resistant *S. aureus* (MRSA) colonization in diabetic foot wounds. Many of these MRSA isolates are becoming multidrug resistant, and are susceptible only to glycopeptide antibiotics such as vancomycin[8]. Low-level resistance to vancomycin is emerging at present[9].

Methicillin-resistant *S. aureus* (MRSA) has become a considerable public health issue during the past decade, due to a significant increase in the incidence of MRSA isolated from patients with complicated infections, including diabetic foot infections of varying severity. Additionally, empiric coverage against MRSA should be considered in institutions with a high MRSA infection rate, or in patients who are at increased risk for MRSA by way of previous MRSA infection, exposure to previous courses of antibiotics, recent hospitalization or nursing home stay, or by close contact with an MRSA patient. Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile becomes necessary in the selection of appropriate empirical treatment of diabetic foot infections. We determined the prevalence of MRSA from pus samples of diabetes patients with foot ulcers and their *in vitro* susceptibility pattern to various antmicrobial agents, to record their current status of MRSA response to commonly used anti Staphylococcus antibiotics in tertiary care hospitals in and around Coimbatore, South India.

Materials and Methods

A total of 7354 pus specimens from diabetic foot ulcer patients were collected for *S. aureus* screening. The samples were obtained from various tertiary care hospitals in Coimbatore and Erode, from Nov 2002 to Oct 2006. All the samples were aseptically handled and processed. Morphotypes were done for all the samples based on the Gram staining method, to determine the organism which was likely to be present. Subsequently, the specimens were inoculated on to Blood agar (BA) plates (aerobic with 5% Co_2), MacConkey's agar (MA) and RCM (Robertson Cooked Meat medium) for further subculturing, and these plates were incubated at 37^0C for 24 hrs. The colonies of Gram-positive cocci in clusters were further confirmed using API-Staph biochemical Strip (bioMeriux, France). All the strains were further tested for the production of free coagulase enzyme using the tube coagulase test, based on standard methods[10]. *Staphylococcus aureus* ATCC 25923, a known coagulase producer, was included as a control strain.

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on the Kirby-Bauer disk diffusion

method, using oxacillin discs (10µg) obtained from Hi-Media Laboratories Pvt Ltd. The isolates were considered methicillin resistant, if the zone of inhibition was 10 mm or less. Further, the antibiotic susceptibility pattern of methicillin resistant *S. aureus* was determined on the day of their isolation by the modified Kirby-Bauer disc diffusion method on Mueller Hinton (MHA) Agar, using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. The antibiotics used were penicillin-G (10 Unit); ampicillin (10µg); cloxacillin (30µg); cephalexin (30µg); erythromycin (15µg); gentamicin (10µg); amikacin (30µg); ciprofloxacin (5µg); ofloxacin (5µg); norfloxacin (10µg); cotrimoxazole (25µg); vancomycin (30µg); linezolid (30µg); netromycin (10 µg) and omnatax (5 µg). Finally the data were recorded and analyzed at the completion of the study as per recommendations of the Clinical Laboratory Standards Institute[11]. *S. aureus* ATCC 29213 was used as a reference strain for the standardization of antibiotic susceptibility testing.

Results and Discussion

Methicillin Resistant *Staphylococcus aureus* (MRSA)

The Mueller-Hinton agar based antibiogram-resistogram pattern study confirmed the presence of 992 MRSA out of 2314 isolates of *S. aureus* obtained from pus samples of diabetes patients with foot ulcers. The remaining strains (1322) were considered as methicillin sensitive *S. aureus* (MSSA). The present study has shown that 42.86 % of *S. aureus* isolates were MRSA. However all the isolates were sensitive to vancomycin, which determined the importance of vancomycin resistant *S. aureus* (VRSA), as it was being increasingly reported[12]. Among the 19 isolates of *S. aureus*, 8 (10.3 %) were methicillin resistant, in a study conducted in a private hospital in Chennai[13]. It is interesting to note that the prevalence of MRSA was found to be 20.83 % in Chennai among diabetic patients[14]. Similarly, in a study of 55 patients in Chennai, 66.6 % of *S. aureus* were found to be methicillin-resistant. Infection of foot ulcers with MRSA was associated with delayed wound healing, compared to ulcers infected with methicillin sensitive *S. aureus* [15].

Previous studies have found that infections caused by MRSA were more common among patients who were previously treated with antibiotics, than in those who were antibiotic naive. Overall, MRSA infections have been associated with increased morbidity, extended hospital stay and increased costs. However, literature on the effect on mortality has been mixed. When it comes to diabetic foot infections, the presence of MRSA may lead to poorer outcomes, an increased risk of amputations, higher treatment costs and increased mortality[16].

In this study, out of 7354 pus samples, 37.82 % was found to be of *S. aureus* isolates, out of which MRSA and MSSA were found to be in the order of 42.86 % and 57.13 %, respectively. The prevalence rate of *S. aureus* and MRSA isolated from pus specimens was compared, as shown in [Table/Fig 1]. Similarly, the comparative analysis of MRSA and MSSA isolates obtained from pus samples of diabetic foot ulcers is shown in [Table/Fig 2].

(Table/Fig 1): Prevalence rate of *S. aureus* and MRSA in diabetic foot ulcers

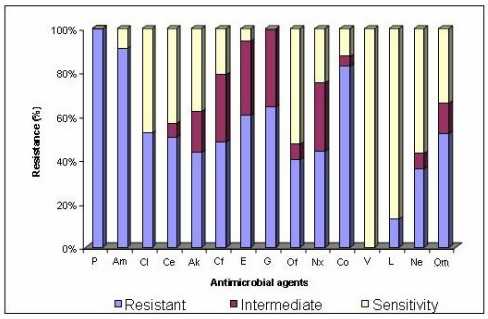
Sample	S. aureus			MRSA		
	Prevalence	95 % Confidence Interval Difference		Prevalence	95 % Confidence Interval Difference	
		Lower	Upper		Lower	Upper
Pus	37.82	35.38	38.25	42.86	40.44	43.27

(Table/Fig 2): Prevalence rate of MRSA and MSSA in diabetic foot ulcers

Sample	MRSA			MSSA		
	Prevalence	95 % Confidence Interval Difference		Prevalence	95 % Confidence Interval Difference	
		Lower	Upper		Lower	Upper
Pus	42.86	40.44	43.27	57.13	54.46	52.67

Antibiogram Pattern for Methicillin Resistant S. Aureus

All the 992 MRSA strains screened from 2314 isolates of *S. aureus,* earlier isolated from pus specimens exhibited different resistant patterns against 16 antibiotics. The diverse drug resistance patterns of MRSA obtained from diabetic foot ulcers is shown in [Table/Fig 3].The percentage of resistance to antibiotics such as penicillin, ampicillin, cotrimoxazole, gentamicin, erythromycin, cloxacillin, omnatax, cephalexin, ciprofloxacin, norfloxacin, amikacin, ofloxacin, netromycin, linezolid and vancomycin was found to be in the order of 100 %, 90.92 %, 82.76 %, 64.11 %, 60.08 %, 52.11 %, 51.91 %, 50.10 %, 48.08 %, 43.75 %, 43.24 %, 39.99 %, 35.88 %, 12.90 % and 0.00 %, respectively. Mehta et al. had reported the percentage of resistance to antibiotics such as penicillin, tetracycline, kanamycin, streptomycin, chloramphenicol and erythromycin to be in the descending order of 88.8 %, 54.4 %, 37.5 %, 28.6 %, 26.3 % and 25 %, respectively. However, no strains were resistant to gentamicin and vancomycin. About 29.0 % of MRSA strains isolated during 1992-1996 from both patients and health care workers and these isolates showed 100 % sensitivity to vancomycin[8]. The antibiogram results in this study suggest that pathogens remain sensitive to a number of agents. Vancomycin was found to be the most effective drug overall against *S. aureus*. These findings are consistent with previous studies. No single antimicrobial agent can cover all of the possible organisms isolated from diabetic foot infections. Our findings illustrate that antimicrobial therapy needs to be selected, based on actual culture findings and antimicrobial sensitivity patterns of isolates.



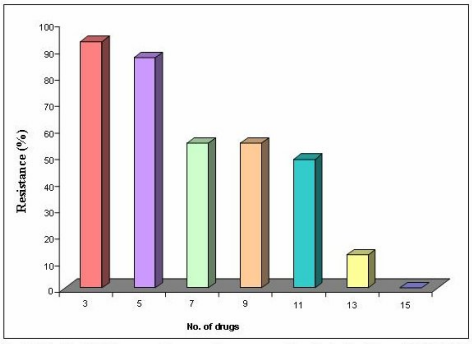
(Table/Fig 3): Antimicrobial pattern among the clinical isolates of Methicillin Resistant Staphylococcus aureus (MRSA)

In one study on spectrum of antimicrobial resistance among MRSA, ciprofloxacin resistance was as high as 90 %, and Qureshi had reported the same as 98.9 %[7]. In contrast, we observed that 48.08 % of the strains were resistant to ciprofloxacin and 39.91 % were resistant to ofloxacin. However, Pulimood has observed only 8 % resistance of MRSA to gentamicin [5] as against 64.11 % in our study. Gentamicin resistance is on the rise since 1996, and an increase of gentamicin resistance from 0 % before 1996 to 80 % after 1996, has been reported[17]. Qureshi et al. had reported a gentamicin resistance of 97.8 %, which is comparatively higher to the current study.

Multiple Drug Resistant Pattern of MRSA

The multi drug resistant pattern for all MRSA isolates is shown in [Table/Fig 4], and it is found that about 55.0 % of the isolates were found to be resistant from 7 to 10 antibiotics, which implies that an alternative antibiotic is the need of the hour to combat MRSA infection associated with diabetic foot ulcers. Although MRSA from diabetic foot ulcers showed higher susceptibility to individual antibiotics when compared with others, we obtained a high percentage of multi drug resistant MRSA from diabetic foot ulcer patients. Majmuder from Assam had reported 23.2 % of the MRSA isolated from clinical specimens to be multidrug resistant [18]. In India, the significance of MRSA has been recognized relatively late, and it emerged as a problem in the 80s and 90s. During the past 15 years, the world wide spread of many such clones

have caused major therapeutic problems in many hospitals, as well as the diversion of considerable resources to attempts at controlling their spread[19]. In this study, all the strains showed susceptibility to vancomycin, and most of them were susceptible to linezolid. As expected, all the strains were resistant to penicillin, and most of them were resistant to ampicillin. But the significant and clinical relevant observation of this study is the moderate resistance shown by MRSA to other conventional antibiotics. The other contemporary reports state higher resistance rates for aminoglycosides and fluoroquinolones.



(Table/Fig 4): Multidrug resistance pattern among the clinical isolates of Methicillin Resistant Staphylococcus aureus (MRSA)

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region, and vancomycin was the only antibiotic found to give uniform sensitivity (100%). When antimicrobials including vancomycin are considered for treatment, choice inevitably requires the need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories. Our study is a preface to enable the epidemiologist to understand the nature of MRSA isolates from diabetic foot ulcers in this part of South India.

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